The American Journal of Surgery (2009) 197, 503–509

The American Journal of Surgery®

Scientific (Exp)/Research

Effects of medicinal mushroom (*Sparassis crispa*) on wound healing in streptozotocin-induced diabetic rats

A.-Hon Kwon, M.D.[a,*], Zeyu Qiu, M.D.[a], Mamiko Hashimoto[b], Kyosuke Yamamoto[b], Takashi Kimura[b]

[a]Department of Surgery, Kansai Medical University, 2-3-1 Shinmachi, Hisakata, Osaka, 573-1191 Japan; [b]Research and Development Center, Unitika Ltd, Kyoto, Japan

KEYWORDS:
Sparassis crispa;
β-glucan;
Wound healing;
Streptozotocin;
Diabetes;
Fibroblasts;
Collagen

Abstract

BACKGROUND: The impaired wound healing in diabetes mellitus is a major clinical problem. *Sparassis crispa* (SC) is a medicinal mushroom and its β-glucan content is more than 40%. This study investigated whether oral administration of SC could improve the impaired wound healing in diabetic rats.

METHODS: Full-thickness skin wounds were created on the backs of streptozotocin (STZ)-induced diabetic rats. Diabetic rats were then divided into 2 groups: SC-treated group that was orally administered doses of 1,000 mg/kg body weight per day of SC for 4 weeks and a control group without SC administration. Moreover, collagen synthesis of purified β-glucan from SC was estimated in vitro.

RESULTS: Wound closure was significantly accelerated by oral administration of SC. Furthermore, in SC-treated wounds there were significant increases in macrophage and fibroblast migration, collagen regeneration, and epithelialization compared with the control group. The levels of type I collagen synthesized by cultured human dermal fibroblasts for the SC group were significantly higher than those for the control group.

CONCLUSIONS: SC can improve the impaired healing of diabetic wounds. This effect might involve an increase in the migration of macrophages and fibroblasts, and β-glucan from SC directly increases the synthesis of type I collagen. Therefore, the use of SC may be extended to the clinical setting and prove an effective promoter of wound healing in patients with diabetes.

The wound healing process can be categorized as follows: inflammation, fibroplasia, neovascularization, collagen deposition, epithelialization, and wound contraction. During the healing process, various growth factors are secreted to accelerate wound healing. It is also accepted that wound repair is an immune-mediated physiologic mechanism.[1] The normal healing process in healthy individuals takes place at an optimal rate, but it is usually delayed or even completely impaired in patients with diabetes. This impaired wound healing in diabetes mellitus is a major clinical problem. High blood glucose hinders proliferation of cells and decreases collagen production.[2] Furthermore, decreased chemotaxis and phagocytosis,[3] a reduction in the levels of growth factors,[4] and the inhibition of fibroblast proliferation all have been suggested to contribute to the observed impairment in wound healing.[5]

Glucan is a natural product, a glucose polymer, which has immune stimulatory activity.[6] Topical or systemic glucan ad-

* Corresponding author. Tel.: +81-72-804-0101 ext. 3115; fax: +81-72-804-2065.
E-mail address: kon@takii.kmu.ac.jp
Manuscript received July 30, 2007; revised manuscript September 28, 2007

0002-9610/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.amjsurg.2007.11.021

ministration enhances wound healing by increasing macrophage infiltration and collagen biosynthesis.[7–11] Moreover, glucans can directly modulate the functional activity of human fibroblasts.[12] *Sparassis crispa* (SC) is an edible/medicinal mushroom and has been eaten in China and Japan. SC was cultivated in Japan recently. At present, many people in Japan eat SC and, to date, there has been no report of adverse events regarding SC.[13] We found that its β-glucan content was 43.5% by the so-called enzyme method. In this study, we investigated whether administration of SC promotes excisional wound healing in streptozotocin (STZ)-induced diabetic rats. Moreover, we examined the effect on collagen biosynthesis in vitro of a branched β-glucan that was isolated from SC.

Methods

Componential analysis of SC

Fruit bodies of SC were cultured by Unitika Co, Ltd, Kyoto, Japan. Following hot air-drying and powdering, the protein, lipid, ash, and carbohydrate content of SC was determined by the Kjeldahl, acid digestion, direct ashing, and high performance liquid chromotography methods, respectively. The β-glucan content was determined by the so-called enzyme method in the Japan Food Research Laboratories.[14]

Animals

Male Sprague-Dawley rats (Charles River, Yokohama, Japan) weighing 180 to 200 g were housed individually in rooms maintained at a constant temperature and humidity, with free access to food and water. They were subjected to a 12-hour light/dark cycle. All experimental animals used in this study were treated according to guidelines set by the Animal Care and Use Committee of Kansai Medical University Animal Center.

Induction of diabetes mellitus

Diabetes was induced by a single 65 mg/kg intravenous injection of STZ (Wako Chemical Co, Osaka, Japan), a toxin specific for insulin-producing cells, in saline-sodium citrate buffer (pH 4.5, Sigma, Inc, St. Louis, MO). Blood glucose levels were measured using a rapid glucometer (GR-102, TERUMO Co, Tokyo, Japan). One week after STZ injection, animals with blood glucose levels above 300 mg/dL were defined as diabetic and used in the study. Diabetic rats were then divided into 2 groups: those orally administered doses of 1,000 mg/kg body weight per day of SC (SC group) for 4 weeks or those without SC administration (control group).

Full-thickness skin wound preparation

Two weeks after STZ administration, diabetic rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (45 mg/kg body weight). The dorsal regions were shaved with an electric clipper and the surgical area was disinfected with 70% alcohol. A round section of full-thickness skin (diameter ~15 mm) was resected with scissors and hemostasis was obtained by direct pressure using sterile gauze. Wounds and surrounding areas were then covered with an adhesive-permeable dressing (Bioclusive; Johnson and Johnson Medical, Skipton, UK).

Estimation of wound healing (wound closure)

Curative effect on the wound (wound closure) was evaluated by tracing the outer margins of the wound on each rat. Wound tracings were scanned using an image scanner (EPSON GT-8000; Seiko Epson Corp, Nagano, Japan), and images were then exported to an image processing program (NIH Image, version 1.52, Public Domain Software). Wound areas were traced manually and calculated in square millimeters. Wound area was measured at 0, 4, 8, 12, 16, and 20 days (10 rats per treatment group per time point) after wounding and the wound closure rate was expressed as the percentage of wound area compared with that on postoperative day (POD) 0 (100%). Rats (n = 5) were killed on PODs 3, 5, 7, and 14 using an overdose of sodium pentobarbital (300 mg/kg intraperitoneally) for histological evaluation.

Histological analysis

The wound and surrounding tissues were fixed with 10% formalin, embedded in paraffin, and sectioned. Sections of 5 μm thickness were stained using the naphthol AS-D chloroacetate esterase technique for neutrophil infiltration[15] and Azan–Mallory for collagen estimation. Macrophages, fibroblasts, vascularization, and epithelialization were examined by immunohistochemical stains. For macrophage staining, anti-macrophage marker mouse monoclonal antibody (NCL- MAC387; Novo Castra Lab, Newcastle, UK), was diluted 1:1,000 in 1% bovine serum albumin (BSA) in .05 mol/L phosphate-buffered saline (PBS, pH 7.5) as the primary antibody, and biotinylated goat anti-mouse IgG (Nichirei Co, Tokyo, Japan) was used as the secondary antibody. Fibroblasts were identified by transforming growth factor (TGF)-β1 staining: anti-human TGF-β1 rabbit polyclonal antibody (Yanaihara, Inc, Shizuoka, Japan) was diluted 1:500 as the primary antibody and biotinylated goat anti-rabbit IgG (Nichirei Co) was used as the secondary antibody. Vascularization was estimated from vessel staining using anti–von Willebrand factor rabbit polyclonal antibody (1:1,200, DAKO, Carpinteria, CA) as the primary antibody and biotinylated goat anti-rabbit IgG (Nichirei Co) as the secondary antibody. Cytokeratin was

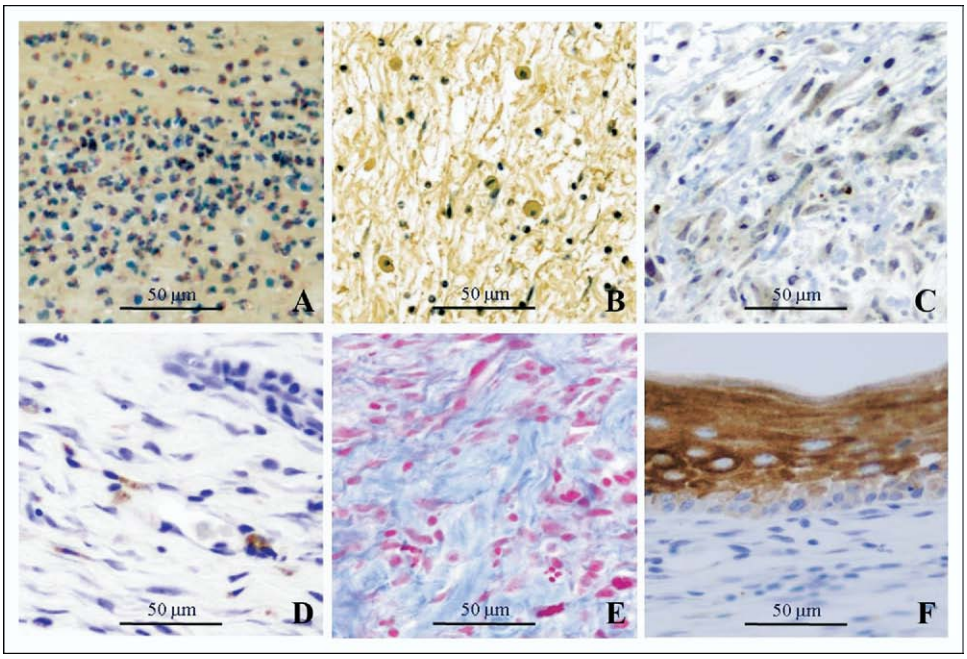

Figure 1 Histological analysis. (A) Neutrophil infiltration detected by naphthol AS-D chloroacetate esterase staining. (B) Macrophage migration (immunohistochemical macrophage staining with anti-cytokeratin, wide spectrum screening rabbit polyclonal antibody). (C) Migrated fibroblasts (immunohistochemical fibroblast staining with anti-human transforming growth factor-β1 rabbit polyclonal antibody). (D) Vascularization (immunohistochemical vessel staining with anti–von Willebrand factor rabbit polyclonal antibody). (E) Collagen regeneration estimated by Azan–Mallory staining. (F) Epithelialization (immunohistochemical cytokeratin staining with anti-cytokeratin, wide spectrum screening rabbit polyclonal antibody).

used as the marker of epithelialization: anti-cytokeratin rabbit polyclonal antibody (1:2,000, DAKO) and biotinylated goat anti-rabbit IgG (Nichirei Co) were used as the primary and secondary antibodies, respectively. The chromogenic reaction was performed with 3-3′-diaminobenzidine-4HCL. Positive staining was indicated by a brown color (Figure 1).

Three separated sections of each wound were examined by light microscopy. The number of neutrophils, macrophages, fibroblasts, and vessels were counted in 5 high-power fields (×100) over 3 separated sections. The stained sections of collagen and cytokeratin were displayed at 40× magnification on a monitor connected to a computer system. The areas stained in blue (collagen) and in blown (cytokeratin) were measured using an image processing program (analysis; Olympus Soft Imaging Solutions GmbH, Münster, Germany).

Extraction of β-glucan from SC

β-glucan was purified enzymatically. Briefly, powdered SC was suspended in .08 mol/L phosphate buffer (pH 6.0) and treated with thermo-stable α-amylase (Sigma) for 30 minutes in boiling water. Then, subtilisin A (Sigma) (30 minutes, 60°C, pH 7.5) and amyloglucosidase (Sigma) (30 minutes, 60°C, pH 4.3) treatments were performed in succession, followed by 80% ethanol precipitation. Precipitate was resuspended in water, and dialyzed against deionized-water. Inner solution was precipitated with ethanol (final concentration 80%) again and dried under reduced pressure. Total sugar and protein contents were measured as 82.4% (phenol-sulfuric acid method) and 3.5% (Bradford method), respectively.

Fibroblast culture

Normal adult human dermal fibroblasts derived from adult skin (NHDF) were obtained from Kurabo, Osaka, Japan. NHDF was cultured with 106S medium supplemented with 2% fetal bovine serum (FBS), 10 ng/mL human epidermal growth factor, 1 μg/mL hydrocotison, 3 ng/mL human fibroblast growth factor-basic (hFGF-B), 10 μg/mL heparin and penicillin-streptomycin-amphotericin B mixture.

Assay of the production of carboxyterminal propeptide of type I procollagen

NHDF-Ad (1×10^4) was incubated with FBS-free 106S medium in a 96-well plate (Iwaki Glass, Tokyo, Japan). After 24 hours, medium was replaced with fresh 106S medium (FBS-free) containing β-glucan preparation. Culture supernatant was collected at 72 hours after sample addition and analyzed for type-I collagen using a carboxyterminal propeptide of type I procollagen (PIP) enzyme immunoassay (EIA) kit (Takara Shuzo, Kyoto, Japan).

Statistical analysis

All data were expressed as means ± SEM. Comparisons among groups were performed using 1- or 2-way analysis of variance (ANOVA), followed by Bonferroni post hoc test when variances across groups were equal or by Dunnett's T3 post hoc test when variances were not equal. Variance equality was tested by Levene statistical analysis. A P value less than .05 was considered to be statistically significant.

Results

Componential analysis of SC

The protein, lipid, ash, and carbohydrate contents of SC were 13.4%, 2.0%, 1.8%, and 21.5%, respectively. The β-glucan content of SC was 43.5%.

Diabetic condition

Blood glucose levels in diabetic rats used in the present study were consistently higher than 300 mg/dL. These high levels were maintained over the entire experimental period. Moreover, oral administration of SC did not affect the blood glucose levels of diabetic rats.

Effect of SC on wound healing

First, the difference in wound healing between normal rats and diabetic rats was measured. Wound contraction in diabetic rats was significantly delayed compared to that in normal rats. In diabetic rats, an administration of SC resulted in rapid wound healing and significant differences were observed between the SC and control groups (Figure 2).

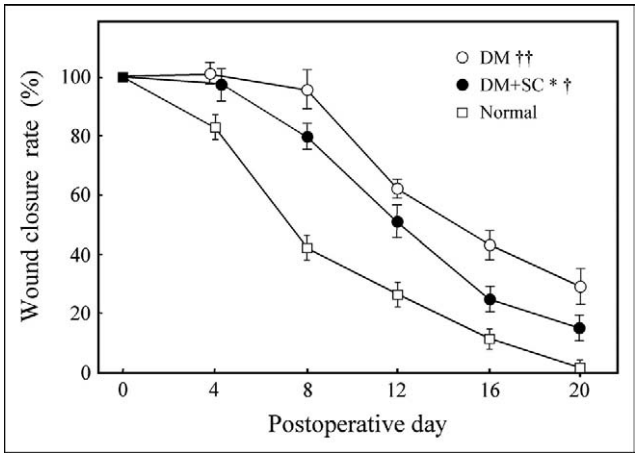

Figure 2 Wound closure in normal rats and diabetic rats treated with *Sparassis crispa* (SC). The wound closure rate was expressed as the percentile of wound area compared with that on postoperative day (POD) 0 (100%). Values are mean ± SEM, n = 10 for each group. *P <.01 vs DM group, †P <.05, and ††P <.01 vs Normal group (2-way ANOVA). DM, streptozotocin-induced diabetic rats; Normal, streptozotocin-untreated rats; POD, postoperative day.

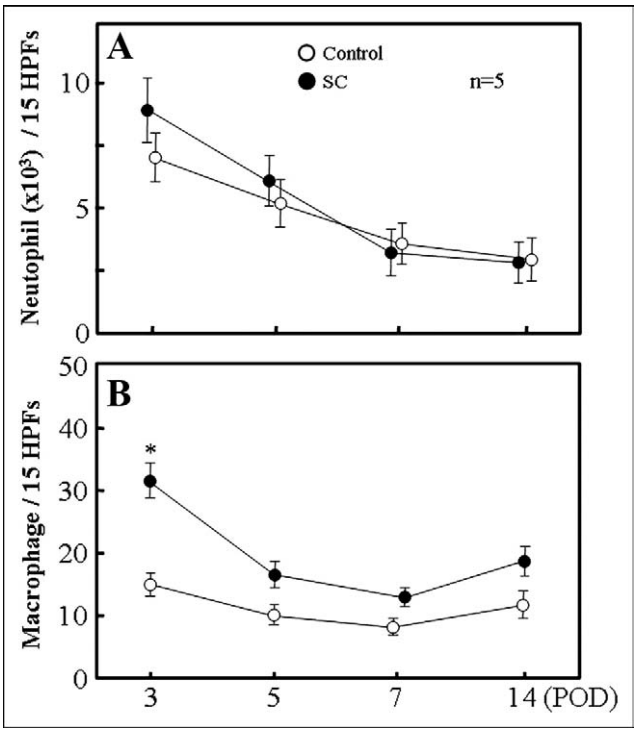

Figure 3 Effect of oral administration of *Sparassis crispa* (SC) on neutrophil infiltration and macrophage migration during skin wound healing in diabetic rats. (A) Number of infiltrated neutrophils stained with naphthol AS-D chloroacetate esterase. (B) Number of infiltrated macrophages (immunohistochemical macrophage staining). Values are means ± SEM, n = 5, for each time point. *P <.05 vs control group (two-way ANOVA). HPFs, high-power fields; POD, postoperative day.

Histological analysis

Administration of SC promoted increases in the number of infiltrating neutrophils on PODs 3 and 5, although without statistical significance (Figure 3A). Conversely, macrophage migration, identified by immunohistochemical staining, was greater in the SC group than in the control group, and a significant difference was seen on POD 3 (Figure 3B). The number of migrated fibroblasts expressing TGF-β1 in the subcutaneous layer after the wounding was significantly greater in the SC group than in the control group on PODs 3 and 5 (Figure 4A). Vascularization identified by von Willebrand factor was slightly enhanced in the SC group; however, there was no significant difference from the control group (Figure 4B). Moreover, the collagen regeneration area estimated by Azan–Mallory staining was significantly wider in the SC group than in the control group on POD 7 (Figure 5A). Finally, the epithelialization area stained for cytokeratin was significantly increased after skin wounding in the SC group compared to the control group on POD 14 (Figure 5B).

Effect of β-glucan from sc on collagen production

As shown in Figure 6, β-glucan treatment dose-dependently increased the PIP concentration in NHDF culture, and statistically significant activity was observed at more than 100 μg/mL.

Comments

The wound healing process can be categorized as follows: inflammation, proliferation (formation of granulated tissue), and tissue remodeling. Re-epithelialization of wounds begins within hours of injury and proceeds first over the margin of the residual dermis and subsequently over granulation tissue.[1,16] Injury to the skin and concomitant blood vessel disruption lead to extravasation of blood constituents, followed by platelet aggregation and blood clotting. These events initiate inflammation and set the stage for repair processes. In response to tissue loss, fibroblasts proliferate and migrate into the defect until the wound is populated by fibroblasts and extracellular matrix.[17]

STZ selectively destroys pancreatic β cells, inhibits the synthesis and release of insulin, and causes the onset of diabetes mellitus.[18] It has been shown that blood glucose levels peak 1 to 3 days after a single high-dose injection of STZ, and then remain elevated.[19] STZ-induced diabetes in rodents is considered to be a model of insulin-dependent

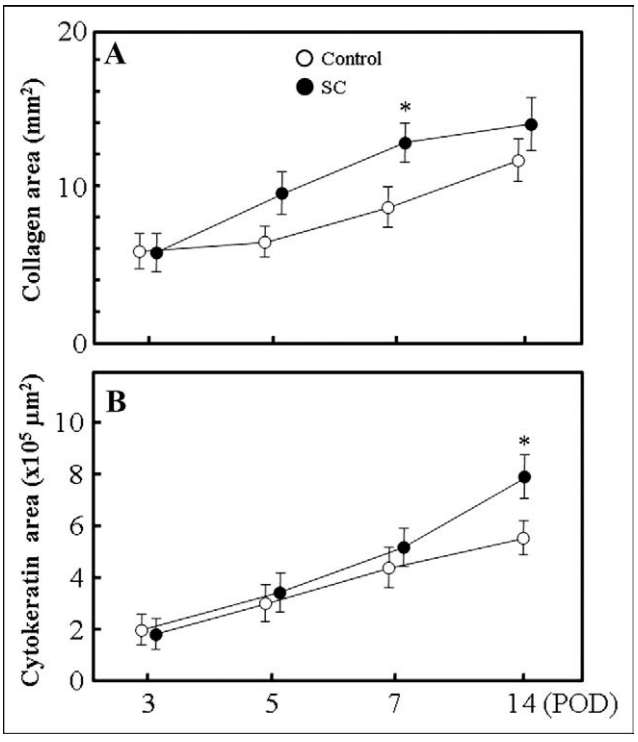

Figure 5 Effect of oral administration of *Sparassis crispa* (SC) on collagen regeneration and epithelialization during skin wound healing in the diabetic rats. (A) Collagen areas (Azan–Mallory staining). (B) Cytokeratin areas (immunohistochemical cytokeratin staining). Values are means ± SEM, n = 5, for each time point. *P <.05 vs control group (2-way ANOVA). POD, postoperative day.

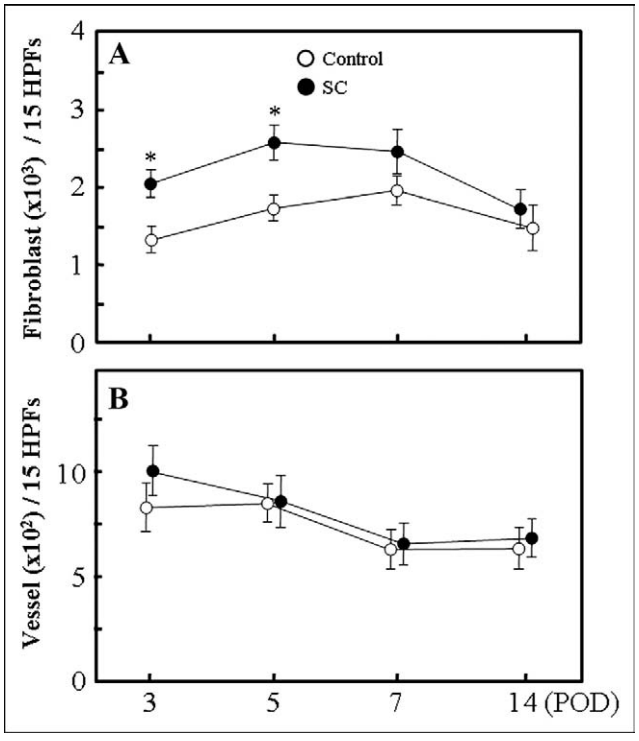

Figure 4 Effect of oral administration of *Sparassis crispa* (SC) on fibroblast migration and vascularization during skin wound healing in diabetic rats. (A) Number of infiltrated fibroblasts (immunohistochemical transforming growth factor-β1 staining). (B) Number of vessels (immunohistochemical von Willebrand factor staining). Values are means ± SEM, n = 5, for each time point. *P <.05 vs control group (2-way ANOVA). HPFs, high-power fields; POD, postoperative day.

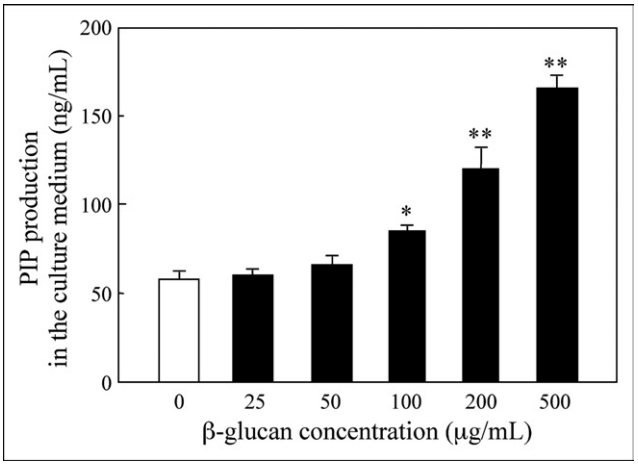

Figure 6 Effect of β-glucan from *Sparassis crispa* on collagen production of normal human dermal fibroblasts. Values are mean ± SEM, n = 3 for each group. *P <.05 and **P <.01 vs β-glucan–untreated group (1-way ANOVA). PIP, procollagen type I C peptide.

diabetes mellitus and is widely used in the study of insulinopenia and hyperglycemia.[20] Diabetes that has been induced by a single high dose of STZ is typically accompanied by diabetic symptoms such as weight loss, polyuria, hyperglycemia, and neuroendocrine dysfunction.[21] Impaired wound healing occurs in patients with diabetes and has been reported to be associated with high blood glucose levels.[22] In the present study, oral administration of SC increased migration of macrophages and fibroblasts, collagen regeneration, and epithelialization under hyperglycemic conditions.

SC was found to be a good source material to prepare the antitumor β-glucan with a very high yield. In a previous study, SC was repeatedly extracted with hot water, cold NaOH, and then hot NaOH, and the primary structure of the polysaccharide fractions of these extracts was analyzed by chemical, enzymic, and nuclear magnetic resonance methodologies. The primary structure of these polysaccharide fractions was found to be 6-branched 1,3-β-glucan, having 1 branch in approximately every third main chain unit. All of these fractions were shown to have strong antitumor activity against the solid form of sarcoma in mice, with strong vascular dilation and hemorrhage reaction by the administration of a microgram quantity.[23] Moreover, β-glucan that was purified from SC enhanced the hematopoietic response in cyclophosphamide-induced leukopenic mice and also induced high amounts of interferon-γ in splenocytes.[24,25]

Previous reports have shown that purified glucans increased macrophage infiltration into the wound milieu, shortened the time to onset of fibroplasia and fibrogenesis, stimulated tissue granulation, and enhanced re-epithelialization in a variety of experimental wound models.[26–28] Multiple pattern recognition receptors for glucans were found on neutrophils, macrophages, fibroblasts, and endothelial cells.[12,29,30] The first step in the modulation of macrophage function by glucan is the binding to a β-glucan–specific receptor on human and murine macrophages.[31] Following binding, glucan is rapidly internalized into macrophages by a very active receptor-mediated endocytosis,[32] and stimulates macrophage cytokine mRNA transcription.[33] Moreover, Kougias et al[12] reported the presence of at least 2 glucan binding sites on normal human dermal fibroblasts, and modulated human fibroblast functional activity by increasing the nuclear factor (NF)-κB and transcription of interleukin-6 mRNA.

After migrating into wounds, fibroblasts produce and deposit large quantities of matrix protein, predominantly types I and III collagen.[34,35] A potential mechanism of glucan-induced early wound repair involves increased wound collagen biosynthesis.[9] In the present study, β-glucan from SC significantly increased type I collagen synthesis in primary cultures of normal human dermal fibroblasts. Type I collagen is the most abundant protein component of granulation tissue.[36] Therefore, assay of the PIP seems to be a useful indicator of human wound healing. Because this propeptide is set free during the synthesis of new type I collagen and stoichiometric release during the formation of fibers, PIP in wound fluid is theoretically considered as a direct measure of collagen type I synthesis.[37] Wei et al[38] also reported that glucan stimulates both type I and III collagen gene and protein expression in a time- and dose-dependent manner, and glucan-induced fibroblast collagen biosynthesis is mediated through a NF-1–dependent mechanism.

In our study, SC was administrated to rats until 3 weeks post wounding. SC administration increased significantly macrophage migration on POD 3, collagen regeneration on POD 7, and epithelialization on POD 14. Macrophage infiltration after injury (inflammation phase, 0–3 days) causes the migration of fibroblasts and release of growth factors. Therefore, SC promotes diabetic wound healing not only in the inflammation phase but also in the subsequent phases of wound healing. For this reason, we did not measure the dynamics of beta-glucan uptake post wounding; however, we should perform further studies.

In conclusion, our results indicated that SC initially increases the migration of macrophages and fibroblasts, which seem to be very important contributing factors to the improvement of diabetic wound healing. Moreover, β-glucan from SC directly increased type I collagen in dermal fibroblasts. Therefore, the use of SC may be extended to the clinical setting and prove an effective promoter of wound healing in patients with diabetes, where skin lesions are often slow to heal.

References

1. Singer AJ, Clark RA. Cutaneous wound healing. N Engl J Med 1999;341:738–46.
2. Hehenberger K, Heilborn JD, Brismar K, et al. Inhibited proliferation of fibroblasts derived from chronic diabetic wounds and normal dermal fibroblasts treated with high glucose is associated with increased formation of l-lactate. Wound Repair Regen 1998;6:135–41.
3. Marhoffer W, Stein M, Maeser E, et al. Impairment of polymorphonuclear leukocyte function and metabolic control of diabetes. Diabetes Care 1992;15:256–60.
4. Beer HD, Longaker MT, Werner S. Reduced expression of PDGF and PDGF receptors during impaired wound healing. J Invest Dermatol 1997;109:132–8.
5. Hehenberger K, Hansson A, Heilborn JD, et al. Impaired proliferation and increased L-lactate production of dermal fibroblasts in the GK-rat, a spontaneous model of non-insulin dependent diabetes mellitus. Wound Repair Regen 1999;7:65–71.
6. Mohagheghpour N, Dawson M, Hobbs P, et al. Glucans as immunological adjuvants. Adv Exp Med Biol 1995;383:13–22.
7. Browder W, Williams D, Lucore P, et al. Effect of enhanced macrophage function on early wound healing. Surgery 1988;104:224–30.
8. Compton R, Williams D, Browder W. The beneficial effect of enhanced macrophage function on the healing of bowel anastomoses. Am Surg 1996;62:14–8.
9. Portera CA, Love EJ, Memore L, et al. Effect of macrophage stimulation on collagen biosynthesis in the healing wound. Am Surg 1997; 63:125–31.
10. Kaiser AB, Kernodle DS. Synergism between poly-(1-6)-beta-D-glucopyranosyl-(1-3)-beta-D-glucopyranose glucan and cefazolin in pro-

phylaxis of staphylococcal wound infection in a guinea pig model. Antimicrob Agents Chemother 1998;42:2449–51.
11. Delatte SJ, Evans J, Hebra A, et al. Effectiveness of beta-glucan collagen for treatment of partial-thickness burns in children. J Pediatr Surg 2001;36:113–8.
12. Kougias P, Wei D, Rice PJ, et al. Normal human fibroblasts express pattern recognition receptors for fungal (1→3)-β-d-glucans. Infect Immun 2001;69:3933–8.
13. Freireich EJ, Gehan EA, Rall DP, et al. Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey, and man. Cancer Chemother Rep 1966;50:219–44.
14. McCleary BV, McCleary BV. Importance of enzyme purity and activity in the measurement of total dietary fiber and dietary fiber components. J AOAC Int 2000;83:997–1005.
15. Yam LT, Li CY, Crosby WH. Cytochemical identification of monocytes and granulocytes. Am J Clin Pathol 1971;55:283–90.
16. Martin P. Wound healing: aiming for perfect skin regeneration. Science 1997;276:75–81.
17. Clark RA. Regulation of fibroplasia in cutaneous wound repair. Am J Med Sci 1993;306:42–8.
18. Elias D, Prigozin H, Polak N, et al. Autoimmune diabetes induced by the beta-cell toxin STZ. Immunity to the 60-kDa heat shock protein and to insulin. Diabetes 1994;43:992–8.
19. Candela S, Hernandez RE, Gagliardino JJ. Circadian variation of the streptozotocin-diabetogenic effect in mice. Experientia 1979;35:1256–7.
20. Rees DA, Alcolado JC. Animal models of diabetes mellitus. Diabet Med 2005;22:359–70.
21. Reagan LP, Magarinos AM, McEwen BS. Neurological changes induced by stress in streptozotocin diabetic rats. Ann N Y Acad Sci 1999;893:126–37.
22. Hoogwerf BJ. Postoperative management of the diabetic patient. Med Clin North Am 2001;85:1213–28.
23. Ohno N, Miura NN, Nakajima M, et al. Antitumor 1,3-beta-glucan from cultured fruit body of *Sparassis crispa*. Biol Pharm Bull 2000; 23:866–72.
24. Harada T, Miura N, Adachi Y, et al. Effect of SCG, 1,3-β-D-glucan from *Sparassis crispa* on the hematopoietic response in cyclophosphamide induced leukopenic mice. Biol Pharm Bull 2002;25:931–9.
25. Harada T, Miura NN, Adachi Y, et al. IFN-γ induction by SCG, 1,3-β-D-glucan from *Sparassis crispa*, in DBA/2 mice in vitro. J Interferon Cytokine Res 2002;22:1227–39.
26. Kenyon AJ, Douglas DM, Hamilton SG. Defective macrophage function in wound repair of P/J mice. Lab Anim Sci 1985;35:150–2.
27. Wolk M, Danon D. Promotion of wound healing by yeast glucan evaluated on single animals. Med Biol 1985;63:73–80.
28. Bhide MV, Dunphy MJ, Mirkopulos N, et al. Promotion of wound collagen formation in normal and diabetic mice by quadrol. Immunopharmacol Immunotoxicol 1988;10:513–22.
29. Mueller A, Raptis J, Rice PJ, et al. The influence of glucan polymer structure and solution conformation on binding to (1→3)-β-d-glucan receptors in a human monocyte-like cell line. Glycobiology 2000;10: 339–46.
30. Wang PL, Azuma Y, Shinohara M, et al. Toll-like receptor 4-mediated signal pathway induced by Porphyromonas gingivalis lipopolysaccharide in human gingival fibroblasts. Biochem Biophys Res Commun 2000;273:1161–7.
31. Muller A, Rice PJ, Ensley HE, et al. Receptor binding and internalization of a water-soluble (1→3)-β-D-glucan biologic response modifier in two monocyte/macrophage cell lines. J Immunol 1996;156: 3418–25.
32. Konopski Z, Fandrem J, Seljelid R, et al. Interferon-γ inhibits endocytosis of soluble animated β-1,3-D-glucan and neutral red in mouse peritoneal macrophages. J Interferon Cytokine Res 1995;15: 597–603.
33. Williams D, Mueller A, Browder W. Preclinical and clinical evaluation of carbohydrate immunopharmaceuticals in the prevention of sepsis and septic sequelae. J Endotoxin Res 1995;2:203–8.
34. Mutsaers SE, Bishop JE, McGrouther G, et al. Mechanisms of tissue repair: from wound healing to fibrosis. Int J Biochem Cell Biol 1997; 29:5–17.
35. Eastwood M, Porter R, Khan U, et al. Quantitative analysis of collagen gel contractile forces generated by dermal fibroblasts and the relationship to cell morphology. J Cell Physiol 1996;166:33–42.
36. Kanzler MH, Gorsulowsky DC, Swanson NA. Basic mechanisms in the healing cutaneous wound. J Dermatol Surg Oncol 1986;12: 1156–64.
37. Haukipuro K, Melkko J, Risteli L, et al. Synthesis of type I collagen in healing wounds in humans. Ann Surg 1991;213:75–80.
38. Wei D, Zhang L, Williams DL, et al. Glucan stimulates human dermal fibroblast collagen biosynthesis through a nuclear factor-1 dependent mechanism. Wound Repair Regen 2002;10:161–8.